ASBMT BEST ABSTRACT AWARDS FOR BASIC SCIENCE

1

Identification of Intestinal Commensal Bacteria Protective Against GVHD in Mice and Humans

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For patients with hematologic malignancies such as leukemias, lymphomas and other related cancers, allogeneic blood/marrow transplantation (allo BMT) is a critically important therapy that can produce cures when chemotherapy alone cannot. More than 25,000 patients undergo allo BMT world-wide each year. A major risk of allo BMT continues to be graft-versus-host disease (GVHD), which results from the donor immune system recognizing the transplant recipient's organs as foreign, leading to life-threatening inflammation. Developing strategies that reduce GVHD but leave global immune function intact should produce a major benefit for patients.

One promising approach that we have developed is targeting the complex community of microbes that reside within our intestinal tracts, collectively termed the intestinal microbiota. While a relationship between the microbiota and GVHD has been suspected for many years, it remains imperfectly understood. Gut decontamination with antibiotics is practiced at some but not all centers, and there is no consensus regarding ideal choice of antibiotic coverage.

Here we present results demonstrating that the abundance of bacteria belonging to the genus Blautia, a commensal commonly found in the intestinal tract of humans, predicts for protection from life-threatening GVHD in allo BMT patients (Figure 1). Furthermore, in murine models, introducing a species of Blautia of murine origin reduces GVHD severity (Figure 2A). It appears to do so by inducing regulatory T cells with generation of short-chain fatty acid metabolic byproducts (Figures 2B and 2C).

Additional studies characterizing the natural history of Blautia abundance in allo BMT recipients demonstrate that the vast majority of patients begin with abundant amounts of endogenous Blautia, but many lose their Blautia in a dramatic fashion during the transplantation process (Figure 3A). Interestingly, loss of Blautia correlates strongly with reductions in oral nutritional intake in both humans and mice (Figures 3B and 3C). Thus development of nutritional intervention strategies to support Blautia abundance following allo BMT could potentially mitigate GVHD. In murine models we have found that these nutritional approaches can successfully prevent loss of Blautia as well as reduce severity GVHD.

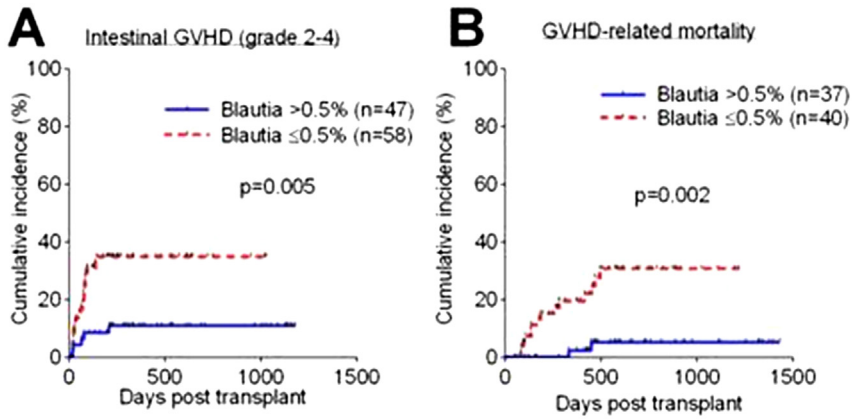

Figure 1. Intestinal Blautia abundance predicts for GVHD in humans, A) A cohort of 105 patients, stratified by abundance of Blautia, was evaluated for incidence of intestinal GVHD, B) The same cohort stratified by Blautia abundance was evaluated for GVHD-related mortality.

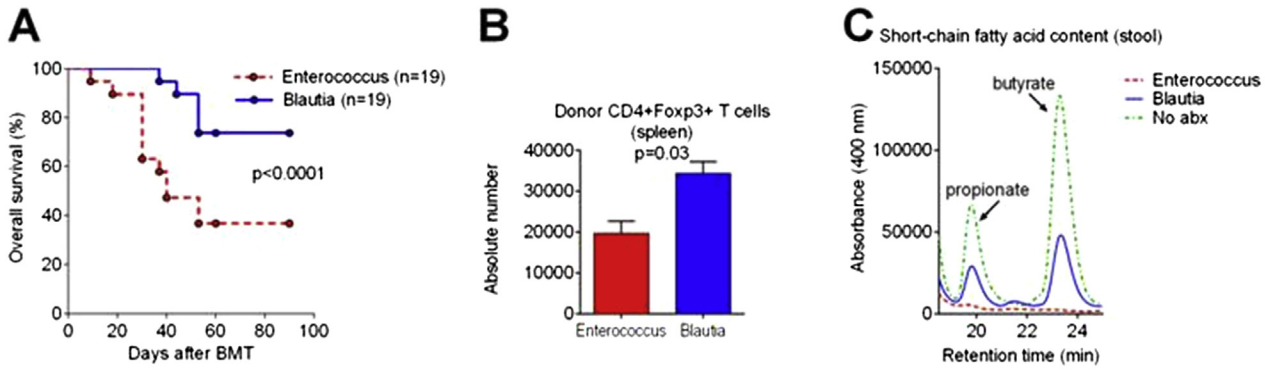

Figure 2. Administration of Blautia to mice mitigates GVHD severity. A) B6 mice were treated orally with a 2 day course of vancomycin, a 2 day course of ampicillin, then inoculated 5 and 7 days later with Blautia or Enterococcus of murine origin, and then 2 weeks later irradiated and transplanted with B10.BR bone marrow and T cells. Presented is overall survival, combined results from 2 experiments. B) Mice were treated as in A. Flow cytometric evaluation of T cell populations, results of a single experiment, mice harvested on BMT day 14 n=4/group. C) Mice were treated with antibiotics and bacteria as in A but without BMT, and stool was evaluated for short chain fatty acid content by HPLC two weeks after bacterial introduction.

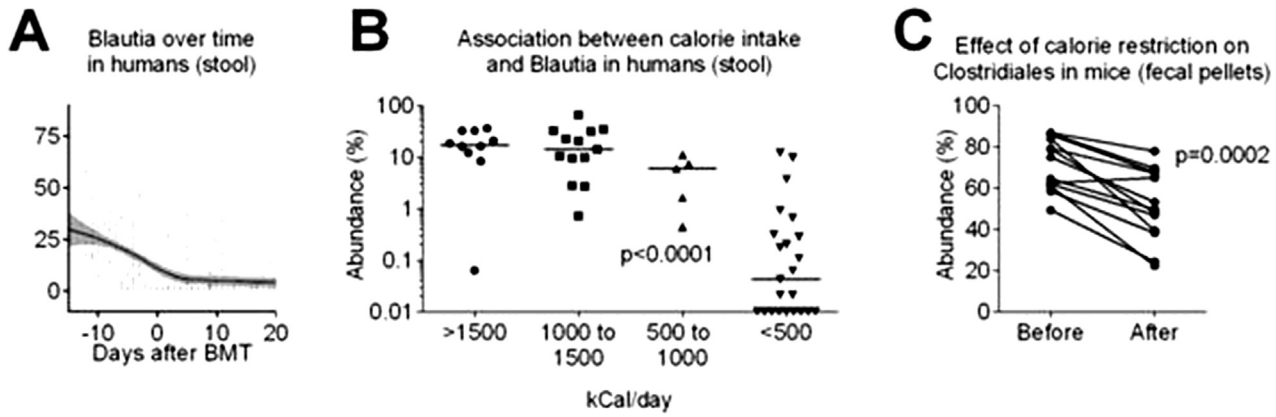

Figure 3. Reduced nutritional intake appears to drive loss of Blautia in allo BMT recipients, A) Ninety-four allo BMT patients were evaluated weekly for changes in the intestinal microbiota during transplant hospitalization. Blautia abundance is depicted by a solid black line with 95% confidence bands shown in gray constructed using moving average filtering. B) Daily nutritional and microbiota analysis was collected in 5 allo BMT patients during transplant hospitalization, and abundance of Blautia analyzed for stool samples stratified by kCal consumption. C) Stool abundance of Clostridials (the order to which Blautia belongs) was assayed in mice before and after one week of 25% reduction m food consumption.

Our results have identified the microbiota as a potent therapeutic target that can be recruited to significantly reduce GVHD. Approaches to potentially translate these findings include investigating the safety of introduction of Blautia to allo BMT recipients, or alternatively developing nutritional strategies to support endogenous Blautia during the transplantation process.

2

$CD4^+$ Invariant Natural Killer T Cells Protect from Acute Graft-Versus-Host Disease Lethality through a Dramatic Expansion of Donor-Derived $CD4^+FoxP3^+$ Regulatory T Cells

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Invariant natural killer T (iNKT) cells are a rare but potent immunomodulatory subset of lymphocytes in humans (TCRα Vα24-Jα18) and mice (TCRα Vα14-Jα18). It has been shown previously that host iNKT cells require interaction with $CD4^+FoxP3^+$ regulatory T cells to protect from acute GVHD after conditioning with total lymphoid irradiation and anti-thymocyte globulin (Pillai et al., Blood 2009). In this study, we investigated the role of highly purified adoptively transferred donor-derived $CD4^+$ iNKT cells in the setting of myeloablative conditioning. Balb/c (H-2Kd) recipient mice were irradiated with 8 Gy and transplanted with T cell-depleted bone marrow together with $1x10^6$ CD4/CD8 T lymphocytes (Tcon) from C57Bl/6 (H-2Kb) donor mice. Mice co-injected with as low as $5x10^4$ highly purified (>99%) $CD4^+$ iNKT cells showed a significant survival benefit compared to mice receiving Tcon alone (p=.002). Consistently, weight and GVHD score improved in mice that received $CD4^+$ iNKT cells. Signal intensity derived from expanding luciferase expressing alloreactive Tcon was significantly lower in animals treated with $CD4^+$ iNKT cells demonstrating inhibition of proliferation of alloreactive Tcon through $CD4^+$ iNKT cells (p<.0001). In vivo CFSE proliferation assay confirmed decreased Tcon proliferation in peripheral lymph nodes (p<.0001), mesenteric lymph nodes (p=.03) and spleen (p=.0005). In addition, Tcon from $CD4^+$ iNKT cell treated mice showed a significantly lower expression of activation markers CD44 and CD25. Interestingly, animals treated with $CD4^+$ iNKT cells showed a robust expansion of donor-derived Neurophilin-$1^{-/low}CD4^+FoxP3^+$ T cells in peripheral lymph nodes, mesenteric lymph nodes, spleen, gut, liver and skin. Moreover, $CD4^+$ iNKT cells derived from $NKG2D^{-/-}$ animals were significantly less effective in preventing acute GVHD lethality (WT vs. $NKG2D^{-/-}$ p=.003). Co-injection of $CD4^+$ iNKT cells did not abrogate GVL reactions of Tcon towards BCL_1 cells measured by bioluminescence imaging (p=ns). With α-GalCer and IL-2 in vitro expanded $CD4^+$ iNKT cells had the same protective effect from lethal acute GVHD compared to freshly isolated $CD4^+$ iNKT cells (p=ns). We conclude that low numbers of highly purified $CD4^+$ iNKT cells protect from lethal acute GVHD in mice through a dramatic expansion of donor-derived $CD4^+FoxP3^+$ regulatory T cells with retained GVL effect. Despite the fact that iNKT cells are a rare cell population, the feasibility of in vitro expansion with retained functionality of $CD4^+$ iNKT cells provide the basis for clinical translation.
EM and RN contributed equally to this study.

3

ATG16L1 Prevents Lethal T-Cell Alloreactivity Mediated By Dendritic Cells

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The role of autophagy in GVHD is unknown. We used a MHC-disparate allo-HSCT model (B10.BR into B6) and compared wild-type (WT) vs $ATG16L1^{HM}$ (hypomorphic (HM) for expression of the ATG16L1 protein) recipients. HM recipients developed significantly increased GVHD mortality (Figure A, $P < 0.001$) and morbidity. This was associated with significantly increased levels of inflammatory cytokines, including TNF-alpha and IL-12 ($P < 0.05$). CFSE analysis demonstrated significantly increased proliferation and activation of alloreactive donor T cells in HM recipients. Alloreactive donor T cells in HM recipients expressed higher levels of LPAM-1,